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THE SPECIAL PHYSICS OF SEGMENTATION AS SHOWN BY THE SYNTHESIS, FROM THE STANDPOINT OF UNIVERSALLY VALID DYNAMIC PRINCIPLES, OF ALL THE ARTIFICIAL PARTHENOGENETIC METHODS.

E. G. SPAULDING.

The genesis of this paper is a twofold one. In the first place the careful perusal of the literature which has appeared in very recent years on the matter of the obtaining of artificial parthenogenesis in various forms by a number of methods, and which includes also varying theories of the process of segmentation as interpretations of these data, this perusal readily convinces one that such a consistent and far-reaching synthetic view of the nature of segmentation as known data would seem to warrant one in trying to obtain is quite lacking. In fact no attempt seems to have been made to show that all the methods employed must result in bringing about one and the same series of physical events in the cell preceding and during segmentation, to which the process resulting from normal fertilization is no exception. That vital (?) phenomena can be reduced to a purely physical basis will doubtless be disputed as long as any details connected therewith remain unstudied or in any way ambiguous. The absence of such a complete reduction is in itself, however, no disproof of the correctness of the view as a theoretical standpoint, and success in it will at least always remain a scientific ideal.[1] A clear and detailed demonstration that the effectiveness of the various artificial parthenogenetic methods can be explained if it is held that one series of physical events always occurs in the process of segmentation would seem therefore to go a considerable way in

[1] The position that this standpoint is logically necessary for biology as a science is discussed in the author's article, "The Contrary and the Contradictory in Biology; a Study of Vitalism," in *The Monist*, July, 1903.

the attainment of that ideal. To attempt to do this states accordingly the purpose of this paper.

The second genetic element was the desire to get such a unitary view as at least a preliminary to and if possible a justification of the attempt to initiate segmentation by new methods, viz., by the application of the electrical current to the unfertilized eggs of the starfish, and although these experiments were unsuccessful, the theory, although based on the experimental work of others, is offered for what it may be worth as an endeavor to gain an end, the value of which in itself will not be denied.

I. Experimental Data.

A brief recapitulation of the results already obtained by artificial parthenogenetic methods may, as a preliminary to subsequent discussion, be pardoned.

In the starfish egg parthenogenesis may be produced by: (1) the use of HCl;[1] (2) increasing the osmotic pressure of the surrounding medium;[2] (3) by lowering the temperature;[3] (4) by mechanical agitation.[4] By the first method it is held that the "parthenogenesis of *Asterias* eggs is to be produced by means of specific (hydrogen) ions," at least this is the interpretation of the fact that 100 c.c. of sea water plus 3–5 c.c. $N/10$ HCl acting for from 3 to 20 minutes on the eggs, which are then removed, brings about the desired result.[5] In the case of the second method,[6] although the results are stated somewhat ambiguously, the maximum number of parthenogenetic eggs seems to have been secured by using 15 c.c. of $2\frac{1}{2}$ N KCl + 85 c.c. of sea water at about 23° C. for 15 minutes, then transferring. As for the third, "eggs of *Asterias* may be made to develop parthenogenetically by exposing them for a definite length of time to a temperature of 1°–7° C., in sea water, and then raising the temperature." As an interpretation of this, we find it stated that "the produc-

[1] Loeb, Fischer u. Neilson, *Archiv für die geschichtliche Physiologie*, Bd. 87, 1901.

[2] Greeley, A. W., Biological Bulletin, IV., 3, Feb., 1903, says that Neilson found this method successful.

[3] Greeley, A. W., *Am. Jour. of Physiology*, VI., 1902, p. 296.

[4] Mathews, A. P., *Am. Jour. of Physiology*, VI., II.

[5] Loeb, Fischer u. Neilson, *loc. cit.*

[6] Greeley, *loc. cit.*

tion of artificial development by lowering the temperature is brought about by an extraction of water from the protoplasm, just as if the eggs had been placed in a solution of higher osmotic pressure than that of the sea water,"[1] though no explanation of the reason for this is offered. A suggestion as to this is however made by Mathews in his comments on the fourth method, that "the getting of parthenogenesis by agitation may be due to a dissolution of the nuclear membrane, since the centrosome originates close to the nucleus, or it may cause the eggs to lose water like the cells of sensitive plants. The loss of water could be caused only by lowering the osmotic pressure in the cell, and this by decreasing the number of molecules in the cell; and this in turn by synthetic processes."[2]

In other forms artificial parthenogenesis may be obtained by similar or slightly different methods; *e. g.*, in *Arbacia* by osmotic pressure, 50 c.c. $\frac{20}{8}$ N $MgCl_2$ or $NaCl$ + 50 c.c. sea water,[3] and at least a segmentation by lack of oxygen, by heat, or by exposure to alcohol, chloroform, or ether;[4] in *Chætopterus* likewise by the use of KCl, KNO_3, K_2SO_4 ($2\frac{1}{2}$ N + 100 c.c. sea water), $NaCl$, $MgCl_2$, $CaCl_2$ and sugar,[5] in *Amphitritus* by $Ca(NO_3)^5$ (2 c.c. N + 99 c.c. sea water);[6] in *Nereis* by osmotic pressure, (20 c.c. $2\frac{1}{2}$ N KCl + 80 c.c. sea water, 30 minutes),[6] in *Podarke obscura* by use of the same solution.[7] As theories and interpretations of the results obtained by these factual methods, we find in addition to those already cited the following, which are quoted in abstract:

"All that the spermatozoön needs to carry into the egg for the process of fertilization are ions, Mg, K, HO or others, to supplement the lack of the one or counteract the effects of the other class of ions in the sea water, or both. The ions and not the nucleins in the spermatozoön are essential to the process of

[1] Greeley, *loc. cit.*

[2] Mathews, *loc. cit.*

[3] Loeb, J., *Am. Jour. of Physiology*, Vol. III., Nos. III. and IX. and Vol. IV. IV.

[4] Mathews, A. P., *Am. Jour. of Physiology*, IV., VII.

[5] Loeb, J., *Am. Jour. of Physiology*, IV., IX.

[6] Fischer, M., *Am. Jour. of Physiology*, VII., III.

[7] Treadwell, BIOLOGICAL BULLETIN, III., 5.

fertilization;[1] or the spermatozoön may carry enzymes." "Either of these two causes affects the most important qualities of life phenomena, *i. e.*, causes the proteids (1) to change their state, or (2) to take up or lose water."[2] Further details as to these two possible events are not given, however, but it is quite evident that the two may be coincident, so that the latter change may take place in any case.

To summarize systematically, a cell division can be caused in various forms by one or more of the following classes of stimuli: (*a*) mechanical; (*b*) heat (or cold); (*c*) osmotic; (*d*) chemical, or, if one will, ionic; the third for the reason that either of the electrolytes MgCl, or NaCl, or the non-electrolyte sugar, may be used for *Arbacia;*[3] therefore no specific chemical effect is to be accepted here. The fourth, a distinctly chemical effect, is evident, for HCl is effective for *Asterias* eggs and KCl is not; so also only the Ca ion for *Amphitritus*. Here then it is the kation that is considered to cause the segmentation, but that a fundamental chemical effect different to an ionic, *i. e.*, electrical charge effect is present is shown by the fact that, keeping the osmotic pressure and the number of charges on the kation the same, but changing the ions, the effect is different. This is confirmed by the comparison of the action of KCl and NaCl on muscle.[4] A specific chemical effect is therefore not done away with even if the difference in effect is reduced to a difference in the path of the charge moving around the atom. For the cause of this latter difference must in turn be a fundamental difference in the atoms themselves. The same kind of proof of an irreducible and ultimate chemical difference is found in the results of Lillie's work on the effect of Na, K, Ca and Mg salts on *Arenicola* and *Polygordius*, and of Mathews on the different stimulating effects on the nerve of NaCl, NaBr, NaI and NaFl. This fundamental chemical difference is related to the difference in solution tension, as Mathew's work this past summer has shown.

However, not alone the stimuli, the external agents initiating

[1] Loeb, J., *Am. Jour. of Physiology*, III., III.
[2] Loeb, J., *Am. Jour. of Physiology*, III., IX.
[3] Loeb, *Am. Jour. of Physiology*, IV., IV. and III., IX.
[4] Loeb, *Am. Jour. of Physiology*, III., VIII.

segmentation are to be put into the above classes, but also, with the addition of the class, surface energy, which is of special importance here, the phenomena taking place within the cell itself, preceding and during cleavage. We accordingly consider the cell to be a physico-chemical object, whatever else it may be, and subject therefore to general physical principles. The special nature of the physical processes that occur in it is to be demonstrated by showing that the effectiveness of the physical methods used for causing segmentation implies that, or at least can be explained, if by each method only one and the same series of events is made to take place. The bringing together of results in this way exemplifies what we have termed synthesis, and the internal agreement with which it is identical makes for the probable correctness of our theory.

2. General Physical Principles to be Observed in the Interpretation of These Data.[1]

If the energies both within and without the cell belong to the classes named, we must in our endeavor to get at the meaning of the data at hand be guided strictly by the most general fundamental chemical and physical principles valid for those. These principles, some of which are of course well known, may be stated as follows:

I. The "first law" of energetics, that of the conservation of energy. This is considered to have an experimental basis in the fact that, *e. g.*, a weight of one kilogram falling 424 meters raises the temperature of one kilogram of water 1° C. as indicated on an arbitrarily selected scale. This is interpreted to mean that the kinetic energy of the falling body is quantitatively equal to the heat energy gained in the rise in temperature. However this cannot be strictly proven, for the two energies are qualitatively different, and have no common factor. It would therefore be

[1] The principles as stated are to be found in no one author, but are with their criticism the result of the study of the works of Planck, Mach, Ostwald, Helm, Wald, Riecke, and others; some of these are as follows: Planck, "Prin. d. Erh. d. Energie"; Ostwald, "Vorlesungen über Naturphilosophie," "Allgemeine Chemie," and other writings; Helm, "Die Energetik nach ihrer gesch. Entw."; Rankine, *Philos. Mag.*, 1867 (4); Mach, "History of Mechanics," "Wärmelehre," "Pop. Lect.," "Analyse der Empf"; Riecke, "Lehrbuch d. Physik."

quite as logical, though not as practical to interpret the two as quantitatively different. To interpret as equal is therefore to base the law of conservation on an assumption not proven, yet not disproven.[1]

In the second place the law is based upon the impossibility of a system's continuing to do work unless energy is received from without. In this the assumption is implicitly contained that work cannot be created *ex nihilo*. Were it possible, however, for a system to receive from without the work (energy) which it itself does, a perpetual motion would be possible.

II. The second law prevents this. In every "Ausgleichung" or transformation (Umgleichung) of energy, some heat is produced, only part of which at best is again available, for the reason that it tends to "dissipate"; it cannot pass from the body of the lower to that of the higher potential (temperature) but only conversely. The entropy of the universe is accordingly said to increase. This characteristic of heat energy is a special case of a law (the second) valid for all the energies. Its meaning is that all events have a definite direction.

According to the first law then if one form of energy disappears another form or forms held to be quantitatively equal to it must appear. Energy may therefore be defined as that which in changing conserves itself. Implicit in both the first and second laws is the definition of it as that which does work, but that there are objections to this is evident from the facts stated in the second, that some energy in the form of heat with no difference of potential (T) cannot do work.

III. The factors of energy. Already present though unrecognized in the early development of mechanics, but made explicit first in thermodynamics, and later extended to all forms of energy is the view that each is made up of the product of two factors, a potential or intensity, on the one hand, and an extensity or capacity factor, on the other. In heat energy these factors are respectively temperature and entropy Q/T (specific heat), in kinetic energy V and MV, in volume energy (gases and solutions)

[1] This procedure illustrates the necessary dogmatism of all science, and the superiority of a pragmatic to a logical justification, a subject which will be treated at length in another paper.

pressure and volume, in surface energy surface tension and surface, in chemical energy chemical intensity (avidity) and mass.

IV. The law of events or action. Every event, *i. e.*, the going over of energy from one body to another or the transformation into another form is conditioned (*a*) not by the absolute quantity of energy involved, but by the potential factors which (*b*) must be opposed to each other in direction and be of different value, *i. e.*, a potential difference must exist; (*c*) this potential difference must not be compensated by a third potential, *i. e.*, must be uncompensated. Unless these three conditions are fulfilled a system remains in its state of equilibrium; if they are given between a system and its environment work is done either on it or by it, the amount of which depends upon the product of the total extensity into the potential difference. If such conditions are held to exist wholly *within* a system so that change occurs in it, this is equivalent to dividing the system into environment and smaller system, the limitation of which in every case is arbitrary though for practical purposes necessary.

V. The *direction* of the energy transfer is always from the higher to the lower potential, the one falling as much as the other rises until equilibrium is reached. By this event however a new potential difference between a second and a third energy form may be created, and with the getting of equilibrium by this a series of events is formed. Assuming the potential of a second energy to have increased or to be *continuously* increasing, the result is that in the "Ausgleichung" between this second and a third within the system, the second may be of the same intensity at the end as at the beginning of this event, while the third shows a rise in potential. This kind of event during which one potential is kept constant is called isocyclic. In comparison to the third the second potential presents here a relative fall. Conversely those events in which the extensity factor remains constant and the intensity alone changes are called adiabatic. Both kinds of changes can be brought about by manipulation of a system which is isolated with the exception of the manipulation. In natural events, however, there is always a change of potential as well as of extensity. The process of getting equilibrium is quite consistent with an absolute rise in two potentials

within a system in relation to a fall in a third potential outside of the system; at the same time the extensity factors of the first two may diminish. Under these circumstances work is done on the system. Conversely, two potentials within a system may get equilibrium and, at the same time, both decrease absolutely in relation to a third external, which rises. The system then does work. Both of its extensity factors may however increase in this process, but this does not necessarily mean a gain in energy, for energy equals potential × extensity.

Concrete instances of these possible cases, which have been selected as bearing directly on our special problem, we shall find in the physical events making up the process of segmentation and cleavage.

3. Surface Tension and Osmotic Pressure.

In the instance of the normal progress of the event of segmentation, *i. e.*, with a cleavage of both nucleus and cytoplasm, no matter whether this is the result of natural or artificial fertilization, it is an undeniable fact that, coincident with and as a culmination of all the processes taking place in the cell there is a decrease in surface tension in at least certain parts of the surface. This follows necessarily from the change in the radius of curvature of the approximately spherical form of the egg to the increased radii of certain parts of the surface of the *constricted* form. For surface tension, the potential of surface energy, varies inversely with the radius of curvature, and is greatest in the spherical form. From this it follows that in cleavage there is in any case an *average* decrease in surface tension accompanying the redistribution of this. The very fact that these changes occur may be advanced as a proof that in protoplasm we are dealing with either a solution or a fluid. Accordingly the surface energy is to be considered as due to or identical with the "attracting forces" (cohesion) of the fluid particles. This redistribution of surface tension may be correlated with and is doubtless confirmed by observed protoplasmic streamings.[1]

It would not, however, occur of itself; it must have an ultimate cause, either within or without the system (the egg), and in either

[1] Cf. Bütschli, "Protoplasm."

case is possible only if it is either the immediate *result* or *cause* of a change in the potential of that energy which is opposed to the surface tension, viz., osmotic, *i. e.*, the "repelling" forces of the substance in solution.[1] The change in surface tension is one part of the "Ausgleichung" of the potential difference existing between it and the potential opposing it, viz., osmotic pressure. The necessary coexistence of these two kinds of energy in a solution and the characteristics of each bring it about however that when one potential decreases the other does also, although there may be a relative fall of one and rise of the other, thus making an isocyclic event possible. For just as the surface tension decreases with an increase in surface and conversely, so also is a decrease in osmotic pressure accompanied normally by an increase in volume (and therefore of surface) and conversely.

It is evident then that in segmentation with its change of form and redistribution of surface tension we are always dealing immediately with the interrelations of two energy forms, surface and osmotic, and mediately with any causes which may act on them, whatever these may be. If we succeed in showing that the various physical agents used for producing artificial parthenogenesis can be effective only by in any case causing changes in either one or both of these energies, then we shall have obtained that unification of evidence which is our purpose.

4. Factors Conditioning Variations and Changes in these Energies.

The existence of an uncompensated potential difference within the egg can be accounted for in two ways. It may be either the result of changes already going on in the egg, *e. g.*, the becoming active of preferments[2] and so the formation of new chemical compounds, processes of maturation, or those leading to "natural death,"[3] but this is the same as saying that such a difference is already there and that it leads to others; or the

[1] In reply to the possible objection that colloidal solutions have no osmotic pressure there is experimental evidence that they have this and are diffusible, thus showing that there is no essential difference between these and other solutions. Cf. Höber, "Physikalische Chemie der Zelle u. Gewebe," s. 43, et seq.

[2] Cf. Hofmeister, "Chemische Organization der Zelle."

[3] J. Loeb, Biol. Bull., Nov., 1902, "Maturation, Death, etc., in Asterias."

result of action between the egg and its environment, in which case the egg may either have done work, as, *e. g.*, in a medium of less osmotic pressure, or work may have been done upon it by means of mechanical agitation, heating, abstraction of water, or fertilization by a spermatozoön. Any of these possible events must take place in complete agreement with the general conditions above outlined, and will in every case of cleavage ultimately condition a change in the relation of two kinds of energy, surface and osmotic.

That this is true is shown by consideration in detail of the characteristics of these two energies. Surface energy is due to the mutual attraction of the molecules of a fluid, which here forms one part, viz., the solvent, of protoplasm. The molecules at a certain distance from the surface are each free to adjust their mean position under the influence of the surrounding molecules, the mean position being that in which each is acted upon equally on all sides, with the result that the mutual attraction is not rendered manifest. At the surface, however, if this be free, or relatively free, as is shown by experiment (*i. e.*, if a chemical difference between medium and egg exists) the molecules are virtually acted upon only by those lying internal to them. The result is a system of forces manifesting their action throughout the fluid and at right angles to the surface, *i. e.*, radially and tending to reduce the surface to the least possible area, *i. e.*, the spherical form. The surface in contact with a chemically different medium so that mixture does not take place, acts like or is in fact a membrane or film.

The factors by which variations in the attraction of these particles for each other are determined are: (1) Their chemical nature, and consequently (2) the density of the fluid, (3) the temperature, a rise in which decreases the tension, (4) the presence of electrical charges.

Osmotic (volume) energy is identical with the mutually repelling forces of molecules in a solvent, and follows the law of gases. Its two factors are accordingly volume and pressure, and in a natural event as the former increases the latter decreases. The conditions upon which variations in this depend are accordingly (1) for equal weights of dissolved substance, and equal

volumes, chemical constitution, (2) nature of solvent, (3) electrolytic dissociation, (4) heat. Changes may be brought by chemical interaction, enzymes, heat, mechanical agitation, or amount of solvent present.

In the egg, which is a system of coëxisting energies, it is then *directly* with the attracting and repelling "forces," *indirectly* with anything conditioning them, that our problem is concerned. To be sure the molecules of both solvent and dissolved substance, the solute, must be admitted each respectively both to attract and to repel. In the former, however, the attracting forces predominate as is shown by the slight effect of temperature; the repelling may therefore be ignored. In the latter the converse is the case, for the same reason.

The form and surface of the egg at any time is accordingly determined directly by these two energies, and indirectly by any of the above enumerated conditions modifying them, for any uncompensated potential differences which may rise through the action of either internal or external causes must be equilibrated with a resultant change in surface and perhaps in volume.

The valid objection may here be offered, however, that a uniform or average decrease in surface tension does not account for the change in form accompanying segmentation. This can doubtless be met by showing that, at the same time that a decrease takes place, a rearrangement also results because of the existence in the egg of localized chemical differences, *i. e.*, the egg is organized. That these exist is shown: (1) experimentally by the different staining reactions of, *e. g.*, the nucleic and cytoplasmic cells to acid and basic dyes;[1] and (2) by observed morphological differences and changes. Granted this chemical organization, localized changes in it and so of the osmotic pressure necessarily present in such a solution can be brought about either normally by the entrance of a spermatozoön, whether its action be enzymatic or chemical, or abnormally by ions, temperature, agitation, or abstraction of water. Differences in osmotic pressure thus arising may remain localized either because of membranes within the egg, *i. e.*, of further chemical differences, or by reason of the relatively slow diffusibility of colloidal particles.

[1] Cf. the researches of Picton and Linder.

The chemical changes in the solute to which they are due may in turn be accompanied by electrolytic phenomena, and so by an attraction of unlike and repulsion of like charges, so that with all of these factors taken together reasonable grounds are furnished for the understanding of the presence of irregularities in the cleavage form. The fact of this constricted form and of the possibility of its explanation on the above basis must be constantly kept in mind in the subsequent considerations. If then the final event in the series of changes leading up to segmentation is that ending with a change of form and average decrease and rearrangement of surface tension, after which a "resting stage" of relative equilibrium exists for at least some time, then this series of events must have resulted from or be in part identical with a disturbance of equilibrium within the cell before the event of cleavage. The various theoretically possible ways in which this disturbance can be brought about must therefore be presented systematically and in detail.

5. Possible Ways of Creating a Difference Between the two Potentials Concerned, Pressure and Tension.

There is, first, the possibility that the surface tension may be greater than the osmotic pressure of the moment can withstand, *i. e.*, that a potential difference in the direction of surface tension — osmotic pressure has been formed. This of course is equalized with the establishment of equilibrium, but therewith both extensity factors, surface and volume must have decreased and the cell have lost water, while both intensities, tension and pressure, however, have presented an absolute increase. A potential difference in this direction might theoretically be caused in two ways, either (1) by increasing the surface tension or (2) by decreasing the osmotic pressure, in each case keeping the other potential constant. Conversely, if the osmotic pressure is first increased, *e. g.*, by an analytic chemical process resulting from oxidation, or if the surface tension is first decreased by, *e. g.*, electrical charges, then a potential difference in the direction of osmotic pressure — surface tension will exist. This being uncompensated, the resulting process of equilibrating is identical with the taking place of an increase in both extensity factors and

a decrease in the intensities; but in the equilibrating of potentials the decrease in the tension is less than in the pressure, for the former is already lower; it therefore presents a relative rise.

This scheme offers a systematic mode of procedure for taking up the examination of the various methods of producing segmentation and development. However, since we are concerned only with the bringing about of an average decrease in surface tension, we shall use only the second class of possibilities.

A. Factors Decreasing Surface Tension Directly.

One theoretically possible way of decreasing the tension directly is by means of either a rise in temperature or by bringing to the surface (the poles of the egg) like electrical charges.

The attracting forces, it has been seen, may be regarded as due exclusively to the solvent, which for protoplasm, we have every reason to believe, is only water; consequently any direct decrease in surface tension by changing the solvent *chemically* seems to be excluded for practical reasons. But a rise in temperature lessens the attraction potential directly at the same time that it increases the osmotic pressure; therewith is equilibrium done away with, work being done on the egg from without. The pressure being too great for the tension, the volume must increase, with absorption of water, until equilibrium is established. The extent of surface therewith increases, but the potential, surface tension, decreases absolutely, although, relative to the pressure opposing it, it rises and the egg thus in turn does mechanical work by displacing the surrounding medium. The *direction* of these events is thus determined by the difference in potential.

With this theoretically possible course of events agrees exactly the observed swelling and liquefaction of cells when heated,[1] as well as the starting of development in *Arbacia* by a rise in temperature, observed by Mathews.[2] This constitutes the first example of factual methods agreeing with our synthetic point of view, and indicates that liquefaction consists either in increasing the pressure by analytic processes, or in absorption of water, or in both.

[1] Greeley, A. W., BIOLOGICAL BULLETIN, IV., 3, 1903; V., 1, 1903.

[2] *Amer. Jour. Physiology*, IV., VII.

Such liquefaction phenomena do not of course in every case mean, nor are they the whole of cell division, neither do they in any case account for this except by also taking into consideration the chemical organization previously referred to. But that such a liquefaction takes place during segmentation is evidenced also by (1) the greater susceptibility of the fertilized egg at that time to ether, HCl, KCl, etc., results obtained by the author in work on *Arbacia*, and an account of which will appear in a subsequent paper, and (2) by the results obtained by Lyon this summer, which indicated a rhythm in the use of oxygen and the giving off of CO_2 by the egg. The last is at the maximum during segmentation, which accordingly points to a greater splitting of molecules at that time and a consequent increase in osmotic pressure and absorption of water.

Another theoretically possible direct cause of a decreased tension would be the presence at the surface of the egg of like electrical charges. But as this concerns our own experimental work we postpone its consideration until further on.

A *fourth* possible method here would be that of mechanical agitation, by means of which the attracting forces of the membrane of the cell would be lessened; for in a physical experiment, at least, the form is charged, the average tension therefore decreased by mechanical work done from without. By analogy this may hold good of the egg, and to it corresponds the method of agitation used on starfish eggs. Its efficacy may be further explained if it is recalled that agitation may also result in a molecular splitting and consequent increase in pressure. As this completes the possible cases under this class of methods we may pass to the consideration of the other class.

B. Factors Directly Affecting the Osmotic Pressure.

We are here concerned with the establishment of a potential difference in the direction of osmotic pressure — surface tension by directly *raising* the former. Two classes of theoretically possible methods for doing this are to be considered, by bringing about (1) analytic chemical changes in the solute and (2) physical changes of pressure.

I. *Chemical Changes in Colloidal Particles by* (*a*) *Chemical Methods.*

Other conditions such as the amount of solvent being assumed as constant, the first class of changes can in theory be caused by any agent which increases the number of molecules or of particles, *i. e.*, which causes a splitting up of these within the egg. For, as is well known, in a given volume the pressure varies directly with the number of molecules. Under these circumstances, in order that subsequently there may be equilibrium between pressure and tension, both potentials must decrease, the latter however presenting a relative rise; at the same time both the volume and surface increase, water is absorbed, and the medium outside the cell is really of smaller volume and greater pressure than before.

This series of events agrees with that which we have seen must take place in segmentation, viz., an average decrease of tension. Confirmatory of the correctness of our theory and quite agreeing with it are a number of experimental methods for obtaining artificial parthenogenesis when the direct effect can be only chemical or ionic or both, but in any case not purely electronic. Thus in *Asterias* eggs HCl is effective, but not KCl, from which a specific H ion effect is inferred.[1] So also are Ca ions alone effective for *Amphitritus.*[2] (The effect of the hydroxyl ion, or of O, can have only the same direct chemical effect primarily; by these conditions segmentation is retarded or exhibited, thus indicating that for segmentation analytic processes are essential; for, *e. g.*, oxygen in the presence of ferments means a splitting up of molecules.) The accepting of a *specific chemical effect* of these ions is not invalidated by the admission of an "electronic" effect also, but is necessitated by just such data as the above, which are the results of an application of the "method of difference." Just how this chemical effect is caused, whether directly by action on the protoplasm, or indirectly by first making certain "preferments" in the egg active cannot be definitely stated, but this deficiency does not in itself alter the correctness of the above view. The effectiveness of another practical method is thus found

[1] Loeb, Fischer and Neilson, *Archiv für geschichtliche Physiologie*, Bd. 87, 1901; previously cited.

[2] Fischer, *loc. cit.*

to be explainable upon the basis of our theory and the usefulness of the attempt to synthesize therewith demonstrated.

From exactly this same standpoint also can the effect of an entering spėrmatozoön be explained, whether its immediate action be chemical or enzymatic, and thus the physical processes of normal fertilization and segmentation made clear, at least in part.

(*b*) *Chemical Changes by* (*b*) *Physical Means.*

Theoretically possible physical means for producing this splitting of particles or of molecules are heat, and mechanical agitation; the former, as a rise in temperature, may be supposed to have this direct effect in agreement with well-known phenomena in pure chemistry, or, as a preliminary to this, to first make certain enzymes in the egg active; or it may be considered to have a purely physical effect, for since $PV = RT$ it directly increases the pressure and decreases the tension. All of these three effects may and probably do coexist. Agreeing with these theoretical possibilities is the practical one of starting development in *Arbacia* by the action of heat (rise in T).

In the same way can also the practical method of causing parthenogenesis, *e. g.*, in *Asterias* eggs by mechanical agitation,[1] be explained, *i. e.*, in analogy to facts in chemistry. For example, in the "diazo-compounds" mechanical shock starts analytic processes. Such compounds are accordingly said to be "metastable." At the same time that the pressure is increased by such analytic processes the potential difference thus formed may be further augmented by a decrease in the tension also caused by agitation, as pointed out above.

The effectiveness of this entire class of methods, both physical and chemical, for causing analytic changes in the solute may be explained as follows: Work is first done on the cell in strict accordance with the general physical principles stated. As a result of the analytic chemical processes taking place, the pressure is at first increased, possibly also the tension decreased at the same time. If the potential difference thus created is uncompensated by a third, *i. e.*, if the pressure of the surrounding medium has been kept constant, *e. g.*, by retransferring the eggs to sea

[1] Mathews, *Am. Journal of Physiology*, VI., 2, 1901.

water in the HCl-*Asterias* method, *i. e.*, if another potential difference exists than in the cell, the latter must in turn do work; its two potentials accordingly decrease, the extensity factors increase, water is absorbed, and as a result of new localized chemical and osmotic differences in the egg plus accompanying electrical changes, the constricted form of cleavage appears.

(*Semipermeable Membranes.*)

In the above presentation the assumption is implicitly made that the surface of the cell acts as a semipermeable membrane, so that, first, only specific ions can enter, and second, others, those within the cell cannot make their exit, while water can pass either way. Only on these conditions can osmotic pressure be truly exerted.

The question accordingly arises, if this possibility is done away with if the so-called membrane is due to, or identical with, only the surface tension of a fluid in which colloidal particles are in solution. The answer to this on the basis of the evidence at hand and to accept the position generally taken is negative, but again brings us to the matter of chemical organization. Rhumbler[1] has shown experimentally that particles in the surface are not freely displaceable, nor do protoplasmic streamings to the outside take place; this can be accounted for only by assuming a foam structure and a state of solution. This means that the apparent membrane is a result of the forces of surface tension. Furthermore, with very probable reasons for the surface being chemically different to the interior as it is to that of the surrounding medium, and with the known fact of the difficult diffusibility of colloidal particles, it is quite intelligible that at the same time that a displacement to the interior is rendered difficult the specific chemical nature of the surface film should prevent the passage through it of some ions while allowing that of others, quite analogous to the artificial semipermeable membranes of Pfeffer. The same explanation would hold good for any other apparent membranes, like that of the nucleus, within the cell whose morphological differentiation can be established.[2] All these factors,

[1] Rhumbler, "Aggregatzustand u. Physikalische Besonderheiten des Zellinhaltes," *Zeitschrift für allg. Physiologie*, I., 3, '02.

[2] Cf. Höber, *loc. cit.*, p. 47, for confirmatory evidence.

viz., local chemical differences, membranes within the cell, difficult diffusibility of colloidal particles, splitting of molecules, localized processes, resulting electrical changes, make the constricted form of the egg at cleavage with its rearrangement and average decrease of tension not only intelligible, but *a priori* probable.

II. Means for Changing the Osmotic Pressure Physically.

Purely physical changes in the repelling forces of the cell are possible, and the theoretical methods to be inferred from these are confirmed by a number of practical methods for producing artificial parthenogenesis.

(*a*) *Hypertonic Solutions.*

The first of these both theoretically and also practically possible methods is the direct raising of the pressure within the cell by first surrounding it with a hypertonic solution and then subsequently transferring it to the sea water whereupon segmentation takes place. This method is used for *Arbacia*, *Asterias*, *Chætopterus*, *Nereis* and *Podarke*, and is perhaps the most important of the artificial parthenogenetic means. Its effectiveness may be explained as follows: In a hypertonic solution there is at first an uncompensated potential difference between the pressure within and without the egg, which is equalized by the passing of water through the membrane of the egg to the medium. The relative number of molecules of solute within the egg is accordingly increased. At the same time with a decrease in the egg's radius of curvature accompanyiug the decrease in size the surface tension has been increasing to compensate the increased internal pressure, though this compensation never quite takes place as long as water continues to be withdrawn, but theoretically is always somewhat behind. The egg thus receives energy from without. With the transferral of the egg to sea water, which is of lower pressure than it is, a potential difference in the opposite direction next exists which is uncompensated by the tension owing to the permeability of the membrane to water; accordingly an event, viz., absorption of water, takes place until the pressure both within and without is the same. In this proc-

ess, however, both the pressure and the tension of the egg *decrease*, as our theory demands, while the volume and surface increase until equilibrium is attained between the two energies within and the pressure without. But therewith new potential differences between these and other energies, *e. g.*, chemical, may have been created, by which the series is continued up through the various stages of development.

However, in this method it is not directly evident why, instead of segmentation taking place, simply the former size of the egg is not recovered when it is returned to sea water. To account for the constriction actually occurring it is again necessary to make the assumption which is nevertheless theoretically justifiable in analogy to well-known phenomena in chemistry, that in connection with localized chemical differences some of the colloidal particles are naturally in a "metastable" condition, which is done away with and chemical changes started by the withdrawal of water, and which therefore cannot subsequently be regained.[1] It may also well be in analogy to known instances elsewhere that so-called preferments become active only on the condition of a certain degree of concentration being present and that, becoming active with the concentration here present, they initiate chemical changes which finally cause the constriction of the cleavage form.

(*b*) *Action of Temperature Changes.*

The second of the class of methods we are here considering, viz., the physical, has to do with variations in temperature. Two possibilities therefore exist, a raising and a lowering of the temperature of the medium and so of the egg.

A raising of the temperature directly increases the pressure, but it may also produce, as has been seen, a molecular splitting either directly or through the mediate action of preferments, and, as before stated, decrease surface tension. Any one or all three of the effects taken together are essential with the formation of a potential difference in the required direction; the starting of development in *Arbacia* eggs by heating may be explained in this way.

[1] Cf. Ostwald, *Vorlesungen über Naturphilosphie*, s. 271 et 353, et seq.

Greeley[1] found however that artificial parthenogenesis could be produced in *Asterias* eggs by exposing the eggs to a temperature of 1°–7° C. and then allowing this to rise, keeping them in the same sea water all the time. This can be explained as follows: A lowering of the temperature directly reduces the osmotic pressure of both medium and egg, yet increases the surface tension. A potential difference within the egg in the direction of tension-pressure is thus created, the equalization of which necessitates a loss of water. This view is confirmed by the spherical form assumed and the losing of water by many species as a result of exposure to low temperatures.[2] The loss of water as a result of the increased contracting forces cannot continue indefinitely for the reason that, by virtue of the increased osmotic pressure caused by it, the egg itself would tend to absorb water. Consequently the *three processes* must be considered to occur until there is equilibrium in the entire "system" of *egg-medium*; likewise they would continue to take place, though in opposite directions, when the temperature was subsequently raised; *i. e.*, the pressure and tension would then both simultaneously decrease and the egg absorb water. Consequently this method would agree with our theoretical demand, that the event of cleavage is always identical with a decrease in surface tension.

(*c*) *Electrical Changes.*

The third possible way of bringing about changes in the egg which are in themselves physical (electrical), but perhaps due directly to chemical causes as we have indicated, is by the use of electrolytic methods. These changes may have to do with both pressure and tension at the same time and we have referred to them previously as perhaps to be necessarily considered as present in any case in order to account for the constricted form of cleavage. For it can be shown that every method that we have analyzed may, at the same time that it results in the changes in chemical composition, pressure and tension, have also accompanying these a change in the electrical condition of the egg.

[1] *Am. Journal of Physiology*, VI., 1902, p. 296.

[2] Greeley, *Am. Journal of Physiology*, 1901, VI., p. 122; BIOLOGICAL BULLETIN, III., p. 165 and V., p. 42.

Lillie[1] in two recent papers has emphasized the importance and essentiality of these electrical phenomena for segmentation, but, inasmuch as he ignores to a certain extent the consideration of the factors we have emphasized, and because we believe it can be shown that his own view is incomplete without this, it may be allowed us to quote quite extensively and in abstract.

Lillie in his first paper finds that "the tendency of colloidal particles to collect at the electrodes indicates that they carry a surface charge, either positive or negative." Precipitation can be caused by ions bearing charges of opposite sign to those of the particles, liquefaction by those of like sign. "The researches of Picton and Linder, and Hardy indicate that the nucleo-proteids especially the chromatin of dividing cells and of spermatozoa are pronouncedly acid and therefore electronegative; cytoplasmic proteids are conversely basic and positive. Accordingly a difference of electrical potential exists between cytoplasm and chromatin in the cell, which difference is greatest at the time of mitosis, when the chromatin is most strongly acid. This potential difference may constitute the primary and determining condition of mitosis."

In confirmation of the correctness of this view he finds that "in all cases the appearance of the cytoplasmic radiations and the formation of the mitotic figure are accompanied by a passage of the nuclear chromatin into a phase rich in nucleic acid. Evidently the two parallel series of changes are intimately connected. Furthermore, the marked resemblance between the rays of the mitotic figure and the electric and magnetic lines of force are additional indications that the process is essentially electrical in nature. The position of the chromosomes during mitosis indicates a mutually repellent action similar to that of similarly charged bodies. The same action takes place in the chromatin filament whereby it assumes a coiled or spiral form."

In his second paper his purpose is to show the necessity of these electrical conditions for segmentation. Evidence for this is that "cytoplasmic cleavage in fertilized *Asterias* and *Arbacia* eggs is prevented in solutions of non-electrolytes, although the

[1] R. S. Lillie, *American Journal of Physiology*, VIII., IV., Jan. 1, 1903, and BIOLOGICAL BULLETIN, IV., 4, March, 1903.

nuclear division continues. In these solutions the electrolytes present in the egg must diffuse outward. Cleavage therefore depends on the presence of these in the cytoplasm. Likewise a strong tendency to fusion shows itself in blastomeres transferred to these solutions during early cleavage stages; therefore further cleavage also depends on the presence of electrolytes in the cytoplasm. This action is due to the ions into which they *dissociate*."

If all this is admitted it seems to us quite necessary to admit or infer therefrom that the presence of electrolytes in the eggs means also the presence of osmotic pressure; one cannot exist without the other; consequently their dissociation is identical with an increase in pressure. This would be in perfect agreement with our own theory, but is quite ignored by Lillie. He does, however, recognize with us the necessity of accepting a decrease and rearrangement of surface tension at cleavage, which he accounts for by a difference of electrical potential between the egg and medium. For "Lippman and Helmholtz have shown that the surface tension is greatest when the electrical potential at the boundary is zero, and decreases as the latter increases, for like electrical charges at the surface oppose the tension and diminish it. From this is evident the importance of electrolytes in segmentation; for the production of a potential difference between the egg and the medium separated by a semipermeable membrane is accompanied only by a migration of ions. Therefore, there are ions within the egg originally, and a potential difference implies that ions of like sign are respectively at the surface and in the interior. This state of affairs is found at cleavage. Observation of the direction of the fibrils in the egg during mitosis agrees with this view, *i. e.*, they correspond with the lines of force.' How is this difference of potential between the surface and the interior established? The answer is that "during segmentation especially is the chromatin markedly acid, the cytoplasm basic." This means in our opinion that there is chemical organization. "Agreeing with the results of Nernst and Olsen, the chromatin represents a charged body by the action of whose negative charges the anions are repelled toward the periphery, or the poles, and the kations are attracted to the nucleus." A curren

passes therefore in the direction of the gradient from periphery to center. Such inductive phenomena resulting from the increased acidity of the nucleus during mitosis must in turn be due, we find, to the preceding chemical changes resulting perhaps from the action of ferments. Accordingly, *it results* that "the center of the astral radiations is the region of highest positive potential, the surface of negative, and hence the decrease in the surface tension by the like charges present."

But a uniform decrease in tension is no change in form ; to bring this about the tension must be unequal. To explain this, Lillie says, "There are indications, *e. g.*, the elongation of the spindle axis, that the primary lowering of surface tension by the above agencies is at the two sides of the egg opposite the astral centers. From the position of the astral centers during metaphase and telophase it is to be expected that the surface negative charges are densest near regions adjoining the long axis of the egg and that there surface tension is lowered to the greatest degree. The effect in these regions will increase as the daughter groups of chromosomes approach the poles, since the inductive action increases as the distance decreases. The surface tension at the regions adjoining the astral centers must therefore decrease as the daughter groups approach the surface, *i. e.*, the difference between the surface tension at the poles and at the equatorial region progressively increases. Eventually the egg is surrounded by an equatorial surface zone possessing higher tension and acting like a constricting band ; a cleavage furrow follows."

"From the fact that not all of these events takes place when the egg is placed in solutions of non-electrolytes, it is clear that cleavage depends on the presence of ions, and that in fertilization the spermatozoön carries either these necessary electrolytes into the egg or ferments which initiate their formation and action."

That the acceptance of the events either identical with or similar to those so ingeniously outlined by Lillie is necessary in order to complete the theory of segmentation advanced in our own paper, may be admitted. But that the two are not contradictory to but rather must supplement each other seems quite as necessary to admit. The constricted form of segmentation must

be accounted for, but if this is done by electrolytic theories, then the existence of osmotic pressure cannot be denied. Lillie, however, neglects its consideration and in this respect his view is incomplete. If the necessity for segmentation of the presence of electrolytes in the egg is *proven* by such data as Lillie has advanced then osmotic phenomena must be also present, and that they play an important part cannot be denied in the face also of the evidence from artificial parthenogenetic methods. Rather, *conversely*, starting with the known fact of the effectiveness of, *e. g.*, the osmotic pressure methods, the possibility of these leading to the electrical changes should be shown. The objection may be made that, although present, osmotic pressure may nevertheless be left out of consideration for the reason that it is of low intensity. This objection does not hold good, we think; for, given a semipermeable membrane and the tendency of surface tension to reach its maximum, the latter will do this until balanced by at least as high a pressure as in the normal surrounding medium, sea water, of the marine forms we are considering.

It therefore seems possible and even necessary to unite the two views; to take the position that the electrical phenomena described by Lillie cannot be done away with but, being essential, they can nevertheless, as we have experimental evidence to show, be brought about in a number of different ways, such as amount of water present, action of heat, agitation, etc.; and that they coöperate with the factors we have emphasized in making up the physical and chemical events of cleavage processes. This possible perfect agreement of Lillie's theory with our own, into which all the factual methods have been shown to fit, adds, it seems to us, one more confirmatory element to the synthesis which it has been our purpose to attain.

The electrolytic methods which have been referred to as the *third* class which bring about physical changes may therefore be explained from the standpoint of this more complete view. As our factual result we have artificial parthenogenesis produced in *Asterias* eggs by the action of $N/10$ HCl solution (2–5 c.c. + 100 sea water) for about one hour, with a subsequent transferral to sea water. Dissociation takes place in the dilute solution used.

The negative results obtained with KCl indicate that it is the H and not the Cl ion that is effective. Two views of the way in which this ion acts are conceivable. First, an interpretation we have referred to previously, it may be that only the H ions penetrate the membrane, either because of *its* specific chemical nature or because of *their* greater diffusion velocity; or, second, that, with no penetration occurring, the cytoplasmic membrane simply attracts the H ions to the surface.

Both views however present difficulties if the H ions are considered to have a purely inductive (physical) action. The first view in its very genesis, because, *firstly*, unless the surface is negative all the time it is hard to understand why the positive ions should even be attracted to it, much less penetrate it; if, on the contrary, it is at first positive they would not reach it, being repelled, unless their velocity of diffusion overcame this repulsion; but *secondly*, because if penetration does occur it is difficult to understand how at least many of the ions can reach the nucleus from which point possibly to, in turn, induce negative charges at the surface; for cytoplasm and H ions are like-charged; these would, however, tend to cause a repulsion of cytoplasmic particles and therefore an increase in pressure. The second view meets with objections because, with *no penetration occurring*, it is difficult to understand also here why, firstly, if the surface is not yet negative before mitosis, either H or any other kations should be attracted, or, secondly, if the surface is negative at all times and the cytoplasm positive, so that the surrounding H ions may induce further negative surface charges, why the H and not other kations as well should be effective. That they are might seem to be explained by the comparative diffusion velocity of H, K and Cl ions (H is 313, Cl 65.9, K 63.8)[1] from which it might be assumed that the K ions are not effective because with them the Cl ions while in the case of HCl the H ions have the greater comparative velocity. But this explanation would not hold good for explaining positive results with $Ca(NO_3)$ on *Amphitritus*,[2] for the diffusion velocity of $Ca_{\frac{1}{2}}$ is 62, that of NO_3 65.[3]

[1] Höber, *loc. cit.*, p. 72.

[2] Fischer, *Am. Jour. of Physiology*, VII., III.

[3] Höber, *loc. cit.*, p. 191.

Between the difficulties of the two views it seems necessary to conclude that *certain* ions penetrate the membrane because of *their* and *its* specific chemical nature and of their greater comparative velocity and notwithstanding the possible repulsion, and that there follows a specific chemical effect on the chemically organized cell contents accompanied by those electrical and osmotic phenomena above considered. That there is a chemical effect is indicated also by the different results obtained in determining the rhythm of immunity of fertilized *Arbacia* eggs to ether, HCl, KCl, etc., an account of which will appear in a later paper. Furthermore, from this standpoint there should be in theory no effect resulting from the use of either the anodal or kathodal end of the current on the eggs of, *e. g.*, *Asterias*, and the negative results of such experiments carried on by the author this summer are confirmatory of this view.

Conclusions and Summary.

Under the experimentally justified assumption that the organism is a peculiar complex of energies, so that general physical principles are therefore valid for it, it is found that the effectiveness of both normal and artificial fertilization methods can be explained from one standpoint, viz., firstly, that the necessary condition for the event of cleavage, is the creation, previous to it, of an uncompensated potential difference between osmotic pressure and surface tension by increasing in a chemically organized egg either absolutely or relatively the pressure or by decreasing the surface tension ; secondly, that the event of cleaving is itself identical with the equilibrating and compensating of this difference, which necessiates an average decrease in both the potentials, osmotic pressure, and surface tension ; and thirdly, that there is an accompanying unequal distribution of electrical charges at the surface and at the center in such a way that constriction results therefrom.

This constitutes the synthesis which we purposed, and is offered only as an attempt, that, although in itself justifiable, presents much that is incomplete and tentative.

College of the City of New York,
Department of Philosophy, December 22, 1903.